

APPENDIX 2

Please amend the paragraph on page 92, line 20, to page 93, line 3, to read as follows:

C1 *See D10*
Prior to library generation, purified DNA can be normalized. DNA is first fractionated according to the following protocol. A sample composed of genomic DNA is purified on a cesium-chloride gradient. The cesium chloride ($R_f = 1.3980$) solution is filtered through a 0.2 μm filter and 15 ml is loaded into a 35 ml OPTISEAL tube (Beckman). The DNA is added and thoroughly mixed. Ten micrograms of bis-benzimide (Sigma; Hoechst 33258) is added and mixed thoroughly. The tube is then spun in a Bti50 rotor in a Beckman L8-70 Ultracentrifuge at 33k rpm from 72 hours. Following centrifugation, a syringe pump and fractionator (Brandel Model 186) are used to drive the gradient through an ISCO UA-5 UV absorbance detector set at 280 nm. Peaks representing DNA from organisms present in an environmental sample are obtained. Eubacterial sequences can be detected by PCR amplification of DNA encoding rRNA from a 10-fold dilution of the *E. coli* peak using the following primers to amplify:

Please amend the paragraph on page 93, line 20 to page 94, line 11, to read as follows:

C2 *See D11*
Plates of the library prepared as described in Example 1 are used to multiply inoculate a single plate containing 200 μl of LB Amp/Meth, glycerol in each well. This step is performed using the High Density Replicating Tool (HDRT) of the Beckman BIOMEK.RTM. with a 1% bleach, water, isopropanol, air-dry sterilization cycle between each inoculation. The single plate is grown for 2h at 37°C and is then used to inoculate two white 96-well DYNATECH microtiter daughter plates containing 250 μl of LB Amp/Meth, glycerol in each well. The original single

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plate is incubated at 37°C for 18h, then stored at -80°C. The two condensed daughter plates are incubated at 37°C also for 18 h. The condensed daughter plates are then heated at 70°C for 45 min. to kill the cells and inactivate the host *E. coli* enzymes. A stock solution of 5 mg/mL morphourea phenylalanyl-7-amino-4-trifluoromethyl coumarin (MuPheAFC, the "substrate") in DMSO is diluted to 600 µM with 50 mM pH 7.5 Hepes buffer containing 0.6 mg/mL of the detergent dodecyl maltoside. Fifty µl of the 600 µM MuPheAFC solution is added to each of the wells of the white condensed plates with one 100 µl mix cycle using the BIOMEK to yield a final concentration of substrate of about 100 µM. The fluorescence values are recorded (excitation=400 nm, emission=505 nm) on a plate reading fluorometer immediately after addition of the substrate (t=0). The plate is incubated at 70°C for 100 min, then allowed to cool to ambient temperature for 15 additional minutes. The fluorescence values are recorded again (t=100). The values at t=0 are subtracted from the values at t=100 to determine if an active clone is present.
